



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of DONALD S. ANSON ET AL.  
Serial Number: 06/839,215  
Filed: March 13, 1986  
For: FACTOR IX PROTEIN

Attorney Docket: 604-8  
Group Art Unit: 183  
Examiner: J. Kushan

DECLARATION UNDER RULE 132

DR. EDWARD G.D. TUDDENHAM, MD, MRCP, MRCPATH., declares as follows:

1. I am Director of the Haemostasis Research Group at the Clinical Research Centre of the UK Medical Research Council, Watford Road, Harrow, Middlesex HA1 3UJ, England. I have been involved in research on haemostasis since 1972. I am a haematologist but have specialised in coagulation and in the biochemistry and molecular biology of haemostasis. I was awarded an MD by the University of London for my thesis on "The structure, function and synthesis of factor VIII" in 1985. I am a co-author of papers on the cloning and expression and structure-function relationships of human factor VIII. I raised the first published monoclonal antibody to factor IX as described in the paper "Preparation of Factor IX deficient human plasma by immuno-affinity chromatography", Goodall, Kense, O'Brien, Rawlings, Rothblat & Tuddenham, *Blood* 59:664-670 (1982). I have also worked on monoclonal antibodies to factor VII and factor XI. I am familiar with recombinant DNA techniques and now direct a research group for the Medical Research Council in which such methods are in routine use. I have published over 50 papers in the area of haemostasis, haemophilia and molecular genetics.
2. I have read the text of the US patent application 06/839,215, the patent examiner's "official action" objecting to the application and his follow-up letter of clarification, and the references mentioned at page 1 lines 20-26 of the patent specification. In the

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light of these references and any other relevant knowledge which I had at the time of 15th March, 1985, I have been asked to answer the following question:

"On the basis of your knowledge and experience in the technical area to which the patent application relates, would you have expected that a biologically active factor IX protein having a specific activity of at least 90% of that of blood-derived factor IX (and free of contamination by pox virus proteins) could be obtained by recombinant DNA means?"

3. On the basis of my knowledge and experience in the technical area to which the patent application relates, I would not have expected that a biologically active factor IX protein having a specific activity at least 90% of that of blood derived factor IX could be obtained by recombinant DNA means using the available cell lines. I regarded this as a highly speculative project in view of the known post-translational modification of the protein requiring highly specific enzyme systems known or presumed to be only present in mature liver, that fully carboxylated and active factor IX could be produced in vitro by a number of different cell lines was a surprise. Of course, a number of groups were working towards this goal in hopes that the problems could be overcome but the fact that they were overcome was not predictable.
4. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patents issued thereon.

E.G.D. Tuddenham

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Date

expected that a biologically active factor IX protein having a specific activity of at least 90% of that of blood-derived factor IX (and free of contamination by pox virus proteins) could be obtained by recombinant DNA means?"

3. My answer is "no". In my opinion, it was not obvious that the rat hepatoma cell line H4-11-E-C3 (Example 1) would produce a properly processed, fully functional factor IX from a transfected DNA plasmid. Although this cell-line was a good choice for these experiments, as it was known to produce active prothrombin, it was by no means assured that the cell would produce active factor IX from a recombinant plasmid. It was also not assured that it could be produced from a dog kidney cell line (Example 2), as such cells were not known to produce vitamin K or to have any of the other functions necessary to convert the precursor protein into functional factor IX.
4. The undersigned further declares that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

  
JANE GITSCHIER

11-17-88

Date